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Commentary

PTEN potentiation of oncolytic HSV therapy for glioblastoma

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The tumor suppressor gene *PTEN* (phosphatase and tensin homolog on chromosome 10) encodes a lipid and protein phosphatase that antagonizes the oncogenic PI3K/AKT signaling pathway. PTEN loss is prevalent in the primary malignant tumor glioblastoma (GBM), reflecting its important role in the pathogenesis of this deadly brain cancer. The N-terminally extended isoform of PTEN (PTEN-Long, PTEN-L), first described by Hopkins and colleagues in 2013,[1] is secreted from cells and enters other cells to exert its phospholipid phosphatase function of the canonical PTEN. In this issue of *Molecular Therapy Oncolytics*, Sahu et al. demonstrate that oncolytic herpes simplex virus (oHSV) therapy of GBM increases GBM stem cell (GSC) features through activation of the IL-6/JAK/STAT3 signaling, which can lead to treatment failure and tumor relapse.[2] The authors' extensive investigations of an oHSV armed with PTEN-L (oHSV-P10) found that oHSP-P10 infection of GBM cells blocked the GSC induction observed with its parental oHSV, oHSV-Q, through suppressing the STAT3 signaling and IL-6 production. This effect was observed in GBM cells regardless of their PTEN status. They further showed that oHSV-P10 was effectively combined with ionizing radiation therapy (IR) to eliminate GSCs and improve therapeutic efficacy in mouse models of GBM. Since GSCs are considered to play a key role in driving GBM recurrence after the standard of care involving radiation therapy and chemotherapy, this work presents a significant translational significance of oHSV-P10 as a potential new GBM therapeutic.

Studies have demonstrated a correlation between STAT3 activation and GSC phenotype induction, which may be promoted by virus infection. The current work showed that oHSV-P10 was able to downregulate STAT3 and IL-6 and reduce GSC phenotypes. STAT3 inhibition or IL-6 neutralization together with oHSV-Q recapitulated the activity of oHSV-P10 alone, indicating the significance of oHSV-P10-mediated inhibition of IL-6/JAK/STAT3 signaling. Interestingly, despite expression of PTEN-L, oHSV-P10 did not fully shut down PI3K/AKT signaling, and the combination of PI3K inhibitor and oHSV-P10 was most effective at eliminating GSCs. These findings indicate the complexity of cellular signaling that influences GSCs. Although PI3K/AKT signaling and STAT signaling are connected, the current work did not elucidate the molecular mechanisms by which PTEN-L or oHSV-P10 inhibited STAT3/IL-6 signaling, which should be a topic of future research.

The authors' mechanistic investigations mostly involved *in vitro* experiments using patient-derived GSC models, identifying the key role of GBM cell-produced IL-6 in oHSV therapy. In GBM, IL-6 positively regulates GSC stemness and immune suppression to promote tumor resistance to therapies. Within the GBM tumor microenvironment (TME), IL-6 is secreted by a variety of cells including tumor cells, GBM-associated macrophages, and endothelial cells. Since PTEN-L is a secreted protein, able to enter adjacent cells and mediate its function, it will be interesting to investigate if and how oHSV-P10 infection could alter IL-6 production by non-GBM cells present in the TME. This question is particularly relevant since the authors' group previously showed that PTEN-L-expressing oHSV-P10 was more effective than oHSV-Q in immunocompetent GBM models due to enhanced elicitation of T cell-mediated anti-tumor immunity.[3] *In vivo*, GSCs and various immune cells interact with each other in the TME, with many interactions favoring tumor progression and evasion of treatment interventions. oHSV-P10-induced IL-6/STAT3 suppression may interfere with some of those to contribute to overall therapeutic benefits.

In the current work, the authors focused on testing oHSV-P10 and its parental oHSV-Q, which is a γ34.5 mutant oHSV. One should note that the specific findings on HSV-Q upregulating GSC signatures may not apply to other oHSVs with differing genetic alterations. For example, G47Δ that carries triple mutations in ICP6, γ34.5, and α47, now approved in Japan for treating GBM patients, is able to replicate and kill GSCs.[4,5] An important unanswered question now is if other oHSVs harboring different genetic mutations would induce GSC phenotypes and IL-6 increase as oHSV-Q was shown to do. It is possible that oHSVs capable of killing GSCs like G47Δ may not activate the IL-6/STAT3 signaling.

The authors demonstrated that oHSV-P10 impaired DNA damage repair mechanisms in infected cells through downregulation of the catalytic subunit of DNA-PK (DNA-PKcs). This feature provided a rationale for combinatorial use of oHSV-P10 and IR. Indeed, oHSV-P10 sensitized intracranial GBM to IR in both human GBM xenografts and murine GBM models. These results have translational implications that oHSV-P10 may be applicable to patients with newly diagnosed GBM for which fractionated radiation therapy is commonly used. So far, combination of oHSV G207, which is genetically very similar to oHSV-Q, and a single dose radiotherapy has been clinically tested in adults and children with malignant glioma (NCT00157703, NCT04482933).[6,7] Because of the capacity of effectively impairing GSCs and the DNA damage response, oHSV-P10 may be able to induce a greater, more durable response to IR, leading to suppression of GBM relapse and improved outcomes. Mechanistically, however, it is

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undetermined how PTEN-L expression further decreased DNA-PKcs levels, while the HSV ICP0 protein has been shown to degrade DNA-PKcs.[8,9] Another limitation was that the therapeutic efficacy of oHSV-P10 and IR was not directly compared with oHSV-Q and IR in an immunocompetent model of GBM. Further studies in immunocompetent models will be needed to confirm the role of oHSV-mediated expression of PTEN-L in radiation sensitization and GSC elimination. Given the reported beneficial impact of oHSV-P10 on the immune TME,[3] oHSV-P10 and IR may be further combined with immune checkpoint inhibitors to design new, mechanistic immunotherapy strategies for targeting GBM.

DECLARATION OF INTERESTS

The authors have no conflict of interests to declare.

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